Storage Temp.
liquid nitrogen
vapor phase

Biosafety Level
1

Please read this FIRST

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: EBTr (NBL-4) (ATCC® CCL-44™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org
800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

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**Subculturing Procedure**

Volumes used in this protocol are for a 75 cm² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
4. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

**Note:** To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.

**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:4 is recommended.

**Medium Renewal:** Twice per week


**Cryopreservation Medium**

Complete culture medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

**Comments**

Previous external testing (Bolin SR et al. Survey of cell lines in the American Type Culture Collection for bovine viral diarrhea virus. J. Virol. Methods 48: 211-221, 1994) as well as early PCR testing at ATCC indicated this culture was negative for bovine viral diarrhea virus (BVDV). However, more recent testing at ATCC has confirmed that this culture has always been positive for BVDV.

**References**

References and other information relating to this product are available online at www.atcc.org.

**Biosafety Level: 1**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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